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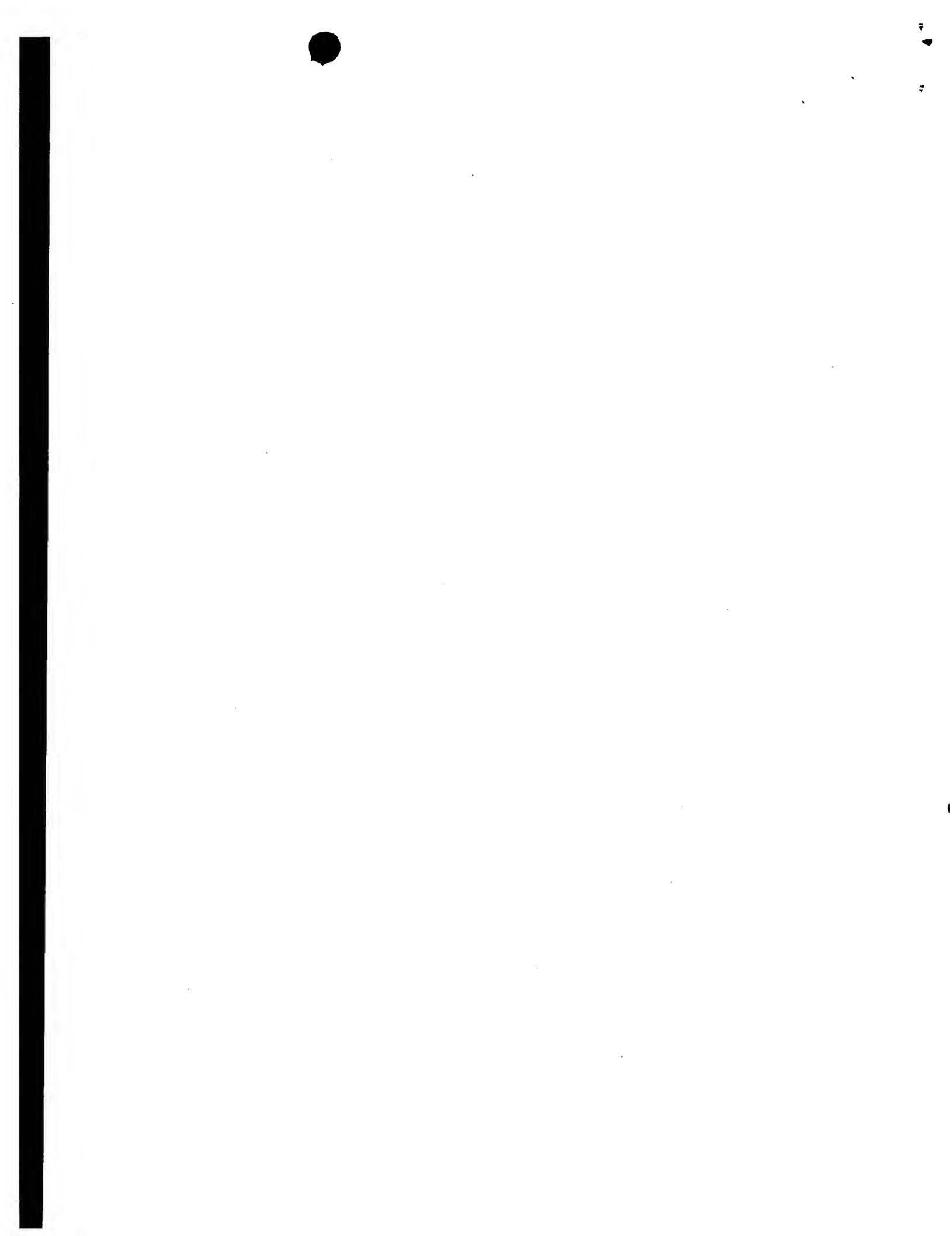
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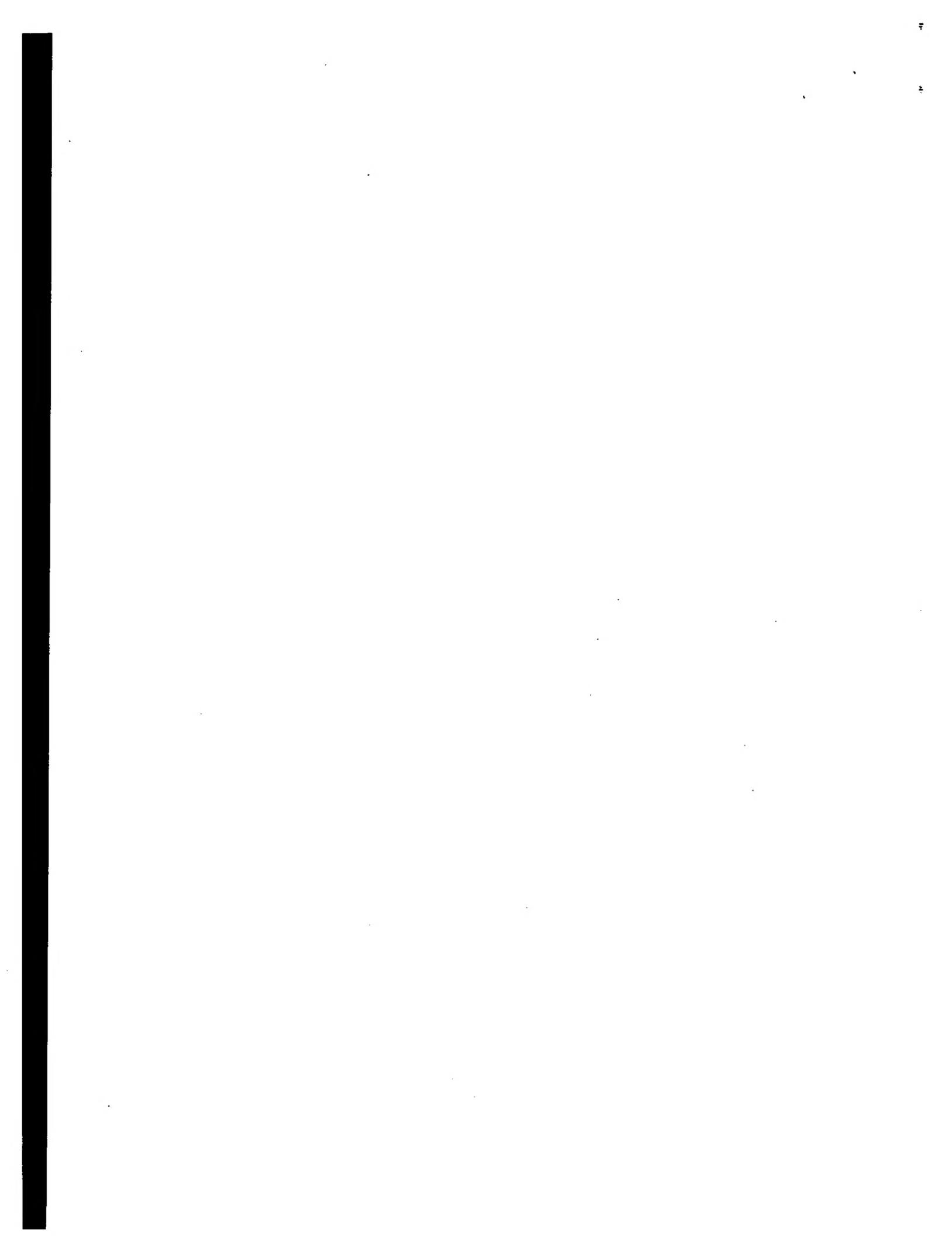
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## TAU AS A MARKER FOR EARLY CNS DAMAGE

### FIELD OF THE INVENTION

The present invention relates to the field of CNS damage. The present invention relates to a new method for the early diagnosis of CNS damage by detection and/or quantification of tau.

### BACKGROUND OF THE INVENTION

The microtubule-associated protein tau exists in different isoforms, of which 4 to 6 are found in adult brain but only 1 isoform is detected in fetal brain. The diversity of the isoforms is generated from a single gene on human chromosome 17 by alternative mRNA splicing (Himmler, 1989; Goedert et al., 1989; Andreadis et al., 1992). The most striking feature of tau protein, as deduced from molecular cloning, is a stretch of 31 or 32 amino acids, occurring in the carboxy-terminal part of the molecule, which can be repeated either 3 or 4 times. Additional diversity is generated through 29 or 58 amino acid-long insertions in the NH<sub>2</sub>-terminal part of tau molecules (Goedert et al., 1989). In vivo tau promotes microtubule assembly and stability in the axonal compartment of neurons by interactions involving its microtubule binding domain which is localized in the repeat region of tau (255-381) (Lewis et al., 1990). In normal circumstances adult brain contains 2 - 3 mol phosphate per mole of tau (Selden and Pollard, 1983; Ksieczak-Reding et al., 1992). Phosphorylation of different sites in normal tau as studied in rat and humans is dependent on the developmental state (Lee et al., 1991; Bramblett et al., 1993; Goedert et al., 1993). Tau variants of 60, 64 and 68 kDa arising as a consequence of phosphorylation have been detected in brain areas showing neurofibrillary tangles (Delacourte et al., 1990; Goedert et al., 1992; Flament et al., 1990, Greenberg and Davies, 1990). These brains contain 6-8 mol phosphate per mol tau (Ksieczak-Reding et al., 1992). In tau isolated from paired helical filaments, phosphorylation can occur at several positions (Iqbal et al., 1989; Lee et al., 1991; Hasegawa et al., 1992). Detection of normally and abnormally phosphorylated tau in brain extracts is done either via antibodies (Mab Alz50: Ghanbari et al., 1990; Mab Ab423: Harrington et al., 1991; Mab AT120 :

Vandermeeren et al., 1993; Mab AT180; Mab AT270 : International application published under WO 95/17429 and Mab AT8 : International application published under WO 93/08302), or via the change in molecular weight (Flament et al., 1990), or else by functional assay (Bramblett et al., 1992). A combination of monoclonal antibodies, each recognizing specific epitopes of tau, has been used to detect the presence of normally and abnormally phosphorylated tau in CSF (Van de Voorde et al., 1995). Tau has been used as a marker to discriminate dementia with altered cytoskeletal properties such as Alzheimer's disease from normal aged subjects or from patients with other types of dementia.

Leukemia is the most common type of cancer in children. During the last twenty years, the survival of children with leukemia has improved markedly based on the routine use of intensive chemotherapy alone or as combined treatment (radiotherapy and chemotherapy). Currently, the estimated overall 10-year survival rate is around 75%. Given the increasing number of childhood leukemia survivors, concern has arisen about long term effects of CNS invasion and of anti-cancer chemotherapy and/or radiotherapy resulting in possible damage to the central nervous system and the need for an early quantitative determination of this CNS damage is increasing. Perinatal asphyxia may be associated with CNS damage as well. To date, clinical, electroencephalographic and neuroradiologic evaluation, together with cerebral blood flow studies are the most readily available methods. However, early and accurate evaluation of the severity of brain damage after a hypoxic-ischemic event, remains one of the most difficult problems in neonatal care.

CNS damage in general may be caused by various causing agents among which anoxia or ischemia, various chemical agents such as pharmaceuticals, physical injuries such as traumas, various disease processes, space-occupying lesions of the CNS and brain invasion induced by different cancers. Current diagnostic procedures for the detection of these brain injuries include lumbar puncture, eye fundoscopy and brain imaging (Raichle, 1998). However, these diagnostic methods only allow detection of the CNS damage in a more advanced stage while already ongoing CNS damage in the early stages cannot be measured by these methods. Therefore, there is an urgent need for a diagnostic method which allows an early detection of CNS damage.

### AIMS OF THE INVENTION

It is an aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms.

It is a more specific aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS including brain tumors, benign or malignant, and brain metastase. It is another more specific aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by invasion of the CNS by leukemia.

It is another more specific aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by invasion of the CNS by lymphoma.

It is another aim of the present invention to provide a method for the detection and/or quantification of CNS damage caused by space-occupying lesions of the CNS, by invasion of the CNS, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms in order to evaluate the effect of treatment of said CNS damage.

It is another aim of the present invention to provide a kit for the diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms.

It is another more specific aim of the present invention to provide a kit for the diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, including brain tumors, benign or malignant, and brain metastase.

It is another more specific aim of the present invention to provide a kit for the diagnosis of CNS damage in an individual, said CNS damage being caused by invasion of the CNS by leukemia.

It is another more specific aim of the present invention to provide a kit for the diagnosis of CNS damage in an individual, said CNS damage being caused by invasion of the CNS by lymphoma.

It is another aim of the present invention to provide a method to screen for compounds which prevent or treat CNS damage.

All the aims of the present invention are considered to have been met by the embodiments as set out below.

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#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for the early in vitro detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms. This method comprises the step of determining and/or quantifying the level of tau and comparing it to the level of tau in control healthy individuals.

10 The present invention relates to the surprising finding that tau levels in CSF samples from children with leukemia are increased compared to upper limit values for healthy individuals. These increased tau levels are an indication of latent central nervous system invasion and already on-going CNS damage long before this CNS damage can be measured by the current diagnostic procedures. Accordingly, tau can be used as an aspecific marker for the early detection of CNS  
15 damage caused by invasion of the CNS by leukemia and in general, as an aspecific marker for the early detection of CNS damage caused by CNS damaging agents such as invasion of the CNS by lymphoma, anoxia or ischemia, chemical agents, physical agents, organisms, space-occupying lesions of the CNS, including primary brain tumors, benign or malignant, brain metastase or a combination of these mechanisms.

20 The term "CNS damage" refers to any condition of the brain which is associated with a neuronal malfunctioning and which is caused by a specific inducing agent or damaging agent. More specifically, CNS damage refers to space-occupying lesions of the CNS which may be caused by any agent including but not limited to primary brain tumors, benign or malignant, brain metastase and parasite derived cysts such as *Taenia solium* or *Echinococcus granulosus*. CNS damage can also be caused by invasion of the CNS by cancer. Cancers that can invade the CNS include but are not limited to leukemia, lymphoma, breast cancer and lung cancer. CNS damage  
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can also be caused by anoxia or ischemia. CNS damage can be caused by chemical agents which include but are not limited to pharmaceuticals, chemotherapy and exposure to chemical compounds. CNS damage can also be caused by physical agents such as traumas or radiation. Organisms that cause CNS damage include but are not limited to prions, viruses, bacteria or parasites. In particular viruses and bacteria that cause CNS damage can be the ones that cause encephalitis or meningitis such as *Neisseria meningitidis* and *Herpes simplex meningoencephalitis*. CNS damage can also be caused by any combination of these causing agents.

The method for the early in vitro detection and/or quantification of CNS damage in an individual can also be used to evaluate the effect of a certain treatment on the CNS damage in said individual. Possible treatments that might influence the status of the CNS include but are not limited to drug treatments, physical therapy, including radiotherapy and gene therapy.

The method for the early detection of the above mentioned CNS damage comprises the step of determining and/or quantifying the level of tau and comparing it to the level of tau in control healthy individuals. The term "tau" as referred to in the present application can be any form of tau, including any state of phosphorylation.

Tau can be detected and/or quantified in vitro by the analysis of the level of tau in a body fluid sample from the patient. In a specific embodiment of the present invention tau is detected and/or quantified in a cerebrospinal fluid sample taken from the patient. In another specific embodiment of the invention tau is detected and/or quantified in a sample of blood derivatives of the patient. Tau can be detected and/or quantified by any method known, including the use of antibodies, the change in molecular weight (Flament et al., 1990), or else by functional assay (Bramblett et al., 1992). In a preferred embodiment tau can be detected by an immunoassay comprising at least the following steps:

- obtaining a sample from the patient; and
- bringing said sample into contact with a monoclonal antibody (primary antibody or capturing antibody) recognizing tau, under conditions being suitable for producing an antigen-antibody complex; and
- detecting the immunological binding of said antibody to said sample.

Advantageously, the monoclonal antibody used in the invention is in an immobilized state on a suitable support. Alternatively, the present process may be put into practice by using any other

immunoassay format known to the person skilled in the art.

The process for the detection of the antigen can then be carried out by bringing together said antigen-antibody complex formed by the antigen and the antibody recognizing tau with:

- a) a secondary antibody (or detector antibody)

\*which can be a monoclonal antibody recognizing an epitope of the antigen-antibody complex but not recognizing the primary antibody alone, or

\*which can be a polyclonal antibody recognizing an epitope of the antigen-antibody complex but not recognizing the primary antibody alone, with said polyclonal antibody being preferably purified by immunoaffinity chromatography using immobilized tau or the tau-primary antibody complex.

- b) a marker either for specific tagging or coupling with said second antibody, with said marker being any possible marker known to the person skilled in the art;

- c) appropriate buffer solutions for carrying out the immunological reaction between the antibodies and the sample on the one hand, and the bound second antibody and the marker on the other hand, and,

- d) possibly also, for standard purposes, a purified or synthetic peptide reactive with the antibodies that recognize tau.

Advantageously, the second antibody itself carries a marker or a group for direct or indirect coupling with a marker.

The term "epitope" refers to that portion of the antigen-antibody complex that is specifically bound by an antibody combining site. Epitopes may be determined by any of the techniques known in the art or may be predicted by a variety of computer prediction models known in the art.

The expression "recognizing", "reacting with", "immunological binding" or "producing an antigen-antibody complex" as used in the present invention is to be interpreted that binding between the antigen and antibody occurs under all conditions that respect the immunological properties of the antibody and the antigen.

Any monoclonal or polyclonal antibodies that specifically recognize tau may be used for the detection of tau. Antibodies specifically recognizing normally and/or abnormally phosphorylated tau include Alz50 (Ghanbari et al., 1990), Ab423 (Harrington et al., 1991), AT8 (International application published under WO 93/08302), AT120 (Vandermeeren et al., 1993); AT180 and

AT270 (International application published under WO 95/17429) and AT100 (International application published under WO 96/04309). But also other antibodies known in the art that specifically recognize tau can be used.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the detection in an individual of CNS damage caused by space-occupying lesions of the CNS including but not limited to primary brain tumors, benign or malignant, and brain metastase.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the detection in an individual of CNS damage caused by invasion of the CNS, specifically invasion of the CNS by leukemia or lymphoma.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the detection in an individual of CNS damage caused by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms.

In a specific embodiment, tau can be used as an aspecific marker for in vivo imaging. Tau can be visualised in situ by non-invasive methods including but not limited to brain imaging methods described by Arbit et al. (1995), Tamada et al. (1995), Wakabayashi et al. (1995), Huang et al. (1996), Sandrock et al. (1996) Mariani et al. (1997). These in vivo imaging methods may allow the localization and visualisation of tau, for example, by use of labelled antibodies recognizing tau.

Tau can also be used as an aspecific marker for in vivo imaging to evaluate the effect of a certain treatment on the CNS damage in an individual. Possible treatments that might influence the status of the CNS include but are not limited to drug treatments, physical therapy, including radiotherapy and gene therapy.

The present invention further relates to a kit for the diagnosis in an individual of CNS damage caused by space-occupying lesions of the CNS including primary brain tumors, benign or malignant, and brain metastase, by invasion of the CNS more specifically invasion of the CNS by leukemia or lymphoma, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms. Any kit that provides a tool for the detection of tau can be used for the diagnosis of the above mentioned CNS damage.

A preferred kit for the diagnosis in an individual of CNS damage caused by space-occupying

lesions of the CNS including primary brain tumors, benign or malignant, and brain metastase, by invasion of the CNS more specifically invasion of the CNS by leucemia or lymphoma, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms is based on an immunoassay and comprises:

- 5      - at least a monoclonal antibody which forms an immunological complex with an epitope of the tau protein;
- a second antibody
  - \* which can be a monoclonal antibody recognizing an epitope of the antigen-antibody complex but not recognizing the primary antibody alone, or
  - \* which can be a polyclonal antibody recognizing an epitope of the antigen-antibody complex but not recognizing the primary antibody alone, with said polyclonal antibody being preferably purified by immunoaffinity chromatography using immobilized tau protein;
- 10     - a marker either for specific tagging or coupling with said second antibody;
- appropriate buffer solutions for carrying out the immunological reaction between the monoclonal antibody of the invention and a test sample on the one hand, and the bound second antibody and the marker on the other hand,
- 15     - possibly a peptide containing one of more tau epitopes for standard purposes.

The present invention also relates to a method to screen for compounds which prevent or treat  
20 CNS damage comprising the step of determining the level of tau and comparing it to the level of tau in control samples.

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these examples are illustrative and can not be construed as to restrict the invention in any way.

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#### EXAMPLES

##### Example 1: Increased tau levels in children with leukemia

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To evaluate the influence of chemotherapy on neuronal damage, a longitudinal study was

conducted, involving 65 children with leukemia (aged 2 to 16 years) without measurable central nervous system involvement, and treated according to standard procedures. A total of 377 CSF samples were analyzed. Before each injection, a small volume of fluid was sampled for routine laboratory analysis and the leftover was used in our study. These children were being diagnosed and then treated for their leukemia at the University Hospital of Leuven, Belgium. Tau protein in cerebrospinal fluid was assessed using the Innotest hTAU (Innogenetics, Zwijnaarde, Belgium).

For all children suspected to have leukemia, a lumbar puncture was performed before the start of the treatment to detect the possible presence of leukemic cells, indicative of central nervous system invasion. The TAU levels measured at that time, and thus before treatment, would serve as the control level to compare chemotherapy-induced changes in the levels of TAU.

In most of the children, the average TAU level at diagnosis was below 250 pg/ml, which is currently considered the upper limit in normal healthy adults. However, we observed that some children with leukemia already had very high TAU levels at diagnosis in spite of the fact that no leukemic cells were detected in the central nervous system. These children constitute a new risk group having brain invasion or leukemia-induced CNS damage, which cannot always be found using current diagnostic procedures (imaging of the brain, lumbar puncture, eye fundoscopy). This was further supported by the increased TAU levels seen in one patient having leukemia with proven cellular invasion into the brain (malignant cells in the cerebrospinal fluid).

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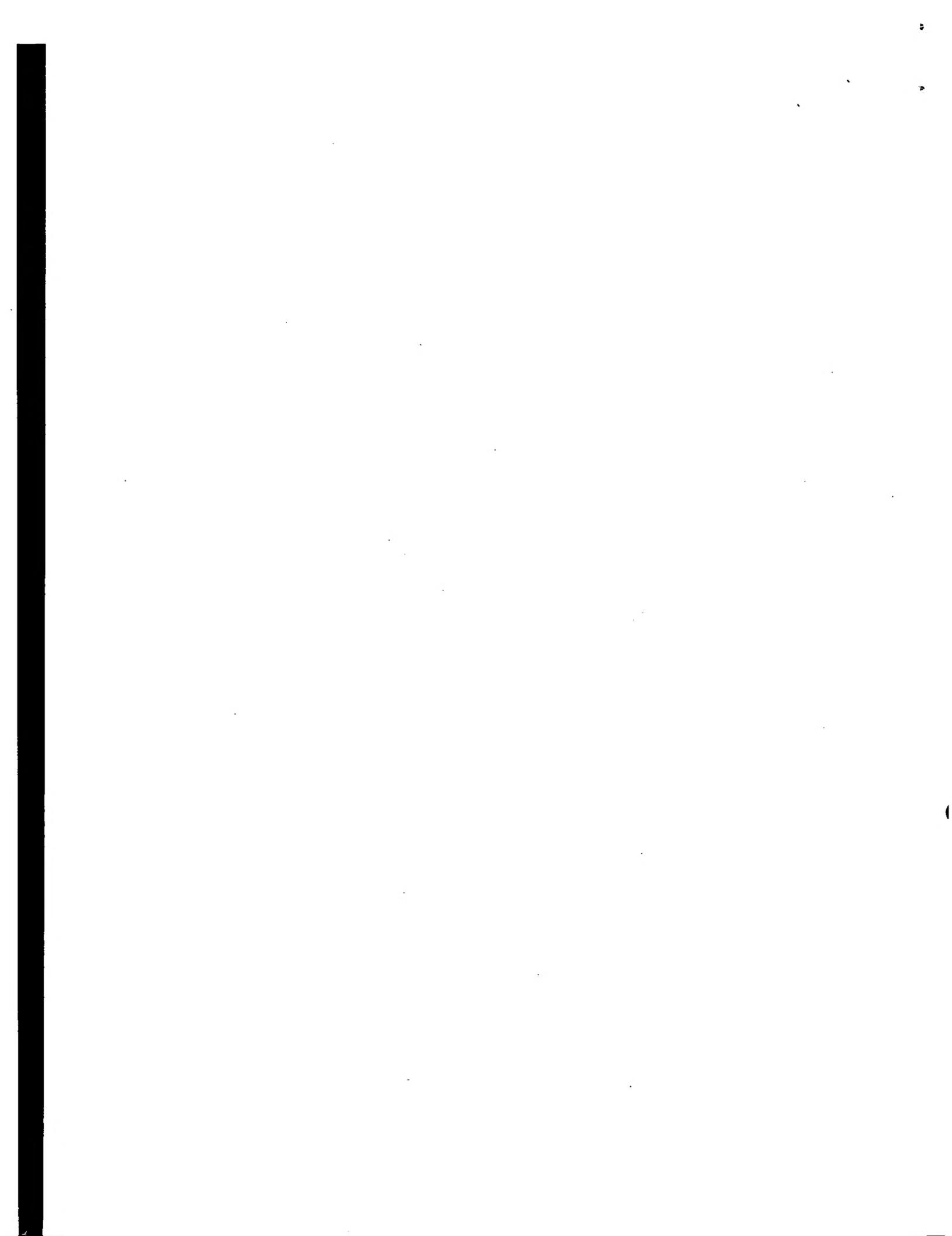
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CLAIMS

1. A method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms, said method comprising the step of determining the level of tau and comparing it to the level of tau in control healthy individuals.
2. A method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms, said method comprising the steps of:
  - obtaining a sample from said individual;
  - determining the level of tau in said sample and comparing it to the level of tau in control healthy individuals.
3. A method according to claim 2 in which the sample is taken from the cerebrospinal fluid of the individual.
4. A method according to claim 2 in which the sample is taken from the blood derivatives of the individual.
5. A method according to claims 1 to 4 in which the space-occupying lesions of the CNS are primary brain tumors, benign or malignant, or brain metastases.
6. A method according to claim 1 to 4 in which the invasion of the CNS is by leukemia.
7. A method according to claim 1 to 4 in which the invasion of the CNS is by lymphoma.
8. A method according to claims 1 to 7 in which CNS damage is detected and/or quantified in order to evaluate the effect of treatment of said CNS damage.

9. The use of tau as an aspecific marker for the manufacture of a diagnostic kit for the detection and/or quantification in an individual of CNS damage caused by space-occupying lesions of the CNS including primary brain tumors, benign or malignant, and brain metastase, by invasion of the CNS, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms.

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10. The use of tau as an aspecific marker according to claim 9 in which the invasion of the CNS is by leukemia or lymphoma.

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11. A kit for the diagnosis in an individual of CNS damage caused by space-occupying lesions of the CNS including primary brain tumors, benign or malignant, and brain metastase, by invasion of the CNS more specifically invasion of the CNS by leucemia or lymphoma, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms comprising a tool for the detection of tau.

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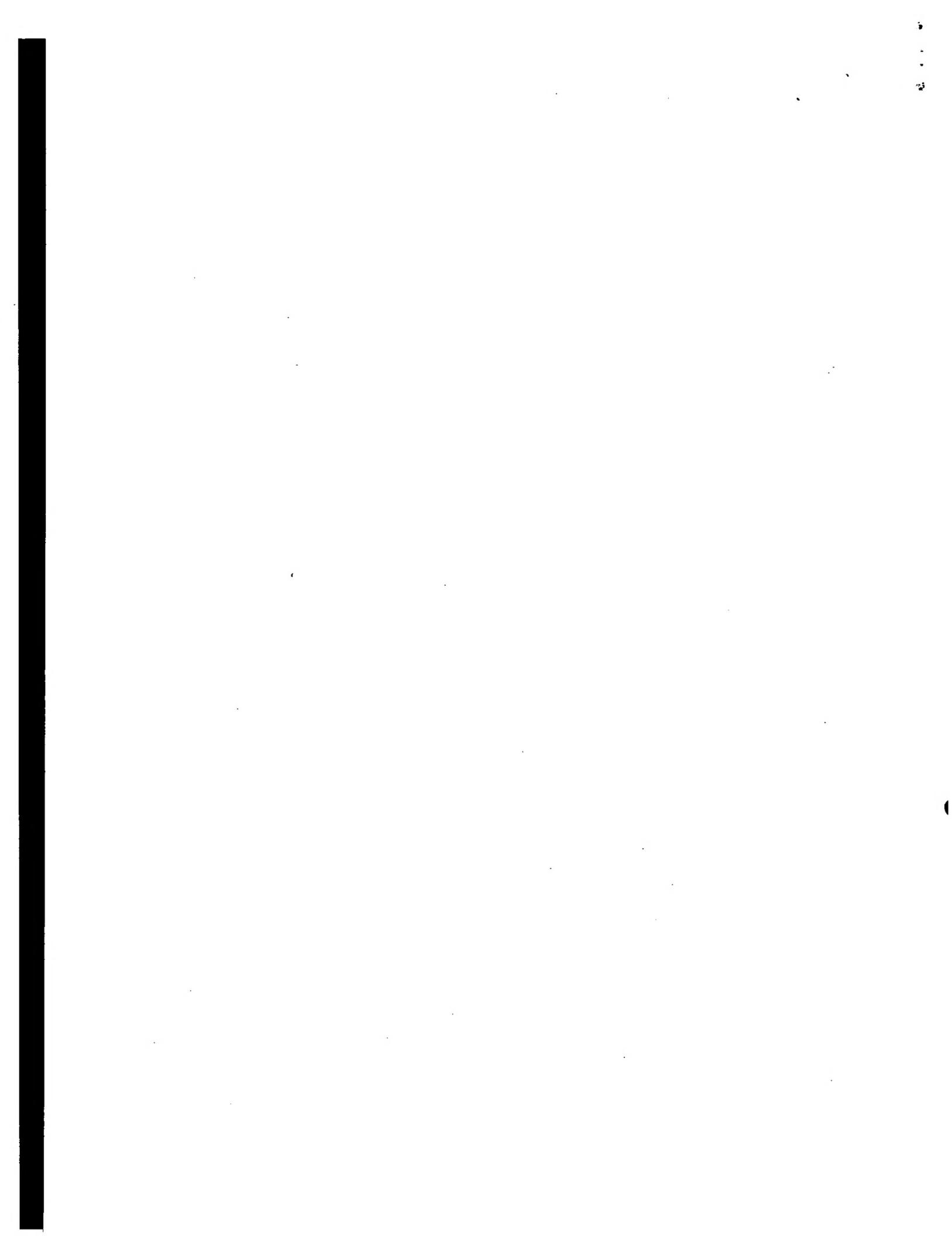
12. A kit according to claim 11 characterized in that said kit comprises:

- a monoclonal antibody which forms an immunological complex with an epitope of tau;
- a second antibody
  - \* which can be a monoclonal antibody recognizing an epitope of the antigen-antibody complex but not recognizing the primary antibody alone, or
  - \* which can be a polyclonal antibody recognising an epitope of the antigen-antibody complex but not recognizing the primary antibody alone, with said polyclonal antibody being preferably purified by immunoaffinity chromatography using immobilized tau protein;
- a marker either for specific tagging or coupling with said second antibody;
- appropriate buffer solutions for carrying out the immunological reaction between the monoclonal antibody of the invention and a test sample on the one hand, and the bound second antibody and the marker on the other hand,
- possibly a peptide containing one of more tau epitopes for standard purposes.

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30 13. A method to screen for compounds which prevent or treat CNS damage comprising the

step of determining the level of tau and comparing it to the level of tau in control samples.



ABSTRACT

The present invention provides a new method for the early diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS including primary brain tumors, benign or malignant, and brain metastase, by invasion of the CNS more specifically invasion of the CNS by leucemia or lymphoma, by anoxia or ischemia, by chemical agents, by physical agents, by organisms or by a combination of these mechanisms. This new method comprises the step of determining and/or quantifying the level of tau in said individual and compairing it to the level of tau in control healthy individuals.

